Restriction Requirement

The office action confirms Applicant's election of claims 30-34 and 46-49, but states that claim 32 has been withdrawn from consideration. Applicants clarify that claim 32 is still pending. If a generic claim is not found to be patentable Applicants will cancel claims directed to non-elected species, including claim 32.

Objection to Claims 30, 31, 33, 34 and 45-49

Claims 30, 31, 33, 34 and 45-49 have been objected to because the claims depend from non-elected claims 1, 8, or 22. Claims 30 and 46 have been amended to incorporate the limitations of the non-elected base claims. The amendment to claims 30 and 46 does not in any way narrow the scope of the claims. Applicants make this amendment solely for the reason that the base claims have been withdrawn from consideration, because the Examiner has asserted that the base claims encompass different inventions. Applicants note that claim 45 was inadvertently included in this list of claim objections. Claim 45 has been withdrawn from consideration.

Rejection of Claims 33, 34, 47 and 48 Under 35 U.S.C. §112, (2)

Claims 33, 34, 47 and 48 have been rejected under 35 U.S.C. §112 as being indefinite. The Examiner maintains that claims 33 and 47 as well as dependent claims 34 and 48 are indefinite in the recitation of "... a method of removing active heparin...". It is unclear to the Examiner what is intended by the term "active heparin". Although Applicants believe that the meaning of the term "active" is clear to those of skill in the art, Applicants have amended claims 33 and 47 to remove the term "active" because it is an unnecessary limitation in the claims. The method can be performed on any heparin, active or inactive. The amendment has not narrowed the scope of the claim in any way.

Rejection of Claims 30, 33, 34, 46, 47 and 48 Under 35 U.S.C. §112, (1)

Claims 30, 33, 34, 46, 47 and 48 have been rejected under 35 U.S.C. §112 "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." The Examiner maintains that the "specification fails

to describe in any fashion the physical and/or chemical properties of the claimed class of substances and identifies only those modified heparinase II enzymes having the amino acid sequence of SEQ ID NO: 2 with either a specific substitution at histidine 440 or cysteine 348, as members of the class of modified heparinases having the necessary functional properties." The Examiner further maintains that the specification does not describe any other species of modified heparinase II enzymes by any characteristics or properties other than having a modified product profile or a k_{cat} value that is at least 10% different than the native product. The Examiner concludes that the claims are so broad that the scope of the claims is not commensurate with the enablement provided by the specification.

Applicants agree that the claims are broad, but disagree that the specification and claims do not teach one of ordinary skill in the art how to practice the invention. The specification adequately and extensively describes the structure of the heparinases through the description of catalytic and binding sites as well as the relationship of these structures to the function of the enzyme. The specification has set forth teachings which describe the regions of the heparinase II molecule which are important for catalytic activity and which can only be replaced with specific amino acids and regions which are not important and can thus, be replaced with non-conservative substitutions. The specification provides extensive detail describing the region of primary and tertiary structure which contribute to the activity of the molecule. The specification also provides working examples demonstrating various mutations which alter the level of enzymatic activity but do not destroy catalytic activity.

The written description of a claimed genus can be satisfied by the actual reduction to practice or by disclosure of identifying, relevant characteristics. For the latter, the disclosure of the invention details the information garnered from extensive experimentation (through biochemical mapping, characterization of the identified sites with kinetic analysis and characterization of the function of modified heparinases). These findings provide adequate guidance to identify to one of skill in the art the active and binding sites of the claimed heparinases as well as the relationship of these sites to the function of the enzyme.

The disclosed invention showed the essential amino acid residues within heparinase II responsible for the binding and catalytic function. The data presented in this disclosure shows that the binding pocket of heparinase II includes two necessary active sites. One which contains cysteine 348 and cleaves heparin and the other which does not contain this residue and cleaves

heparan sulfate. Additionally mapping studies also indicated an additional residue, which is another active site, histidine 451. Heparinase II residues Cys 164 and Cys 189 were found to be non-critical to enzyme activity and can be substituted or modified without affecting the activity.

Experiments which consisted of chemical modification, proteolytic mapping studies and site directed mutagenesis found that histidine residues are essential amino acids for the catalytic activity of heparinase II. These experiments demonstrated that at least histidines 238, 451 and 579 are essential for activity. The results from the mapping studies were corroborated from site directed mutagenesis experiments as well as biochemical experiments. Further, mutants of His 252, His 347 and His 440 were shown to have differential activities towards heparin and heparan sulfate. The results of these experiments demonstrated the ability to manipulate the sequences of heparinases to alter the binding ability with respect to heparin and heparan sulfate. The binding residues of heparinase II were also detailed. These residues are provided in the specification and are residues 446-451 (the heparin-binding sequence). Mutations at these residues would result in modified heparinase II molecules with increased or decreased activity with respect to heparin.

Additionally, not only are the structure and functional properties of these heparinases detailed in the specification but several representative species are also disclosed. These include heparinase II molecules with mutations at the following residues: Cys 348, His 252, His 347, His 440, His 238, His 451 and His 579. These modified heparinases were disclosed in the specification and the resulting enzymatic activity explained. For example, mutation of Cys 348 decreased enzymatic activity of heparinase II with regard to heparin making it react exclusively on heparan sulfate, mutation of His 252, 347 or 440 resulted in differential activity with regard to both heparin and heparan sulfate. The mutation of His 440 resulting in decreased activity to heparin but similar activity to heparan sulfate as that of native heparin. Finally, mutation of His 451, 238 and 579 produced modified heparinase II molecules with reduced activity to heparin and heparan sulfate.

The Examiner has stated that Applicant has not taught one of skill in the art how to make modified heparinases having a modified heparinase k_{cat} value or a modified product profile which differs from that of the native heparinase II having a substitution at His 440 or Cys 348. Applicants respectfully disagree. The specification provides a detailed description of the structure of the heparinase II molecule and how the structure relates to the function of the

enzyme. Molecular biological techniques are well-known to those of ordinary skill in the art. Using the teachings found in the instant specification, one of ordinary skill in the art would be able to produce modified heparinases having altered activities, which results in a reduced enzymatic activity or an altered product profile without undue experimentation. The level of skill in the art is well-developed. One would simply need to follow the guidance set out in the specification. The specification provides extensive direction and guidance as well as working examples. In view of this, it would not require undue experimentation from a skilled practitioner to make a modified form of heparinase which still retains activity.

The technology to produce these modified heparinases and assess their enzymatic activity is well within the skill in the art. For instance, the k_{cat} value may be determined by enzymatic activity assays. As provided in the specification on page 18, a reference for an assay for measuring k_{cat} was given (Ernst, S. E., Venkataraman, G. Winkler, F., Godavarti, R., Langer, R., Cooney, C. and Sasisekharan, R. (1996) Biochem. J. 315, 589-597). In addition to analyzing the modified k_{cat} value, modified product profiles can also be determined by methods known in the art. A preferred method was cited on page 21 from Rhomberg, A.J. et al., PNAS, V. 95, p. 4176-4181 (April 1998). Other methods were also disclosed on page 22 for assessing the product profile. These methods include total UV absorbance, viscosity, mass spectrometry or capillary electrophoresis.

The Examiner also maintains that the disclosure is limited to modified enzymes having the amino acid sequence of SEQ ID NO: 2 with substitution at either His 440 or Cys 348 as the specification did not teach which modifications the protein would be tolerant or intolerant of. The Examiner also argues that detailed knowledge of the protein's structure as it relates to its function was also not given. Applicants respectfully disagree. The disclosure provides information about the structure as it relates to the function of heparinase II through details of the active binding sites as well as information regarding the activity of representative mutant enzymes. Modified heparinase II molecules with a mutation at cysteine 348, a residue that is involved in heparin binding, were shown to have reduced enzymatic activity with respect to heparin. Molecules with mutations at histidines 451, 238 and 579 were shown to have modified enzymatic activity with respect to heparan sulfate. Mutations at histidine 440 resulted in reduced enzymatic activity with respect to heparin but nearly the same activity (as native enzyme) with respect to heparan sulfate. Additionally, the specification provided numerous examples of

possible amino acid substitutions such as those listed on pages 19-20. Therefore with all of the information presented in the disclosure as well as the skill of those in the art, the results of the modifications of the enzymes are predictable, and the breadth of the claims is supported.

In conclusion, Claims 30, 33, 34, 46, 47 and 48 are directed to modified heparinase II molecules having a modified k_{cat} value or a modified product profile which differs from native heparinase II. The specification provides a detailed description of the structure of heparinase II as well as how the structure relates to the function of the enzymes. As described above examples of the regions of the protein structure that may be modified without altering the function of heparinase II are indeed given, contrary to the Examiner's assertions. The numerous examples demonstrate the tolerance/intolerance of the enzyme's function to modification through the elucidation of the active and binding sites and the description of the critical residues. Additionally, the specification outlines the necessary steps and procedures to produce modified heparinases and to test their resultant activity.

The disclosure provides sufficient guidance to modify the heparinases to elicit the desired effect through the knowledge given of the active and binding sites, the specific examples of mutants and the lists of possible substitutions. This information clearly provides guidance to one of skill in the art with a reasonable expectation of success. In light of the specification one of skill is enabled to produce modified heparinases by standard recombinant technology utilizing the description of the active and binding sites of native heparinase II as well as standard methods known in the art to determine the altered product profiles and/or k_{cat} value with respect to the native enzymes. Examples of these techniques are given in the specification and many more are known in the art. In view of this, it would not require undue experimentation for one of skill to practice this invention.

The specification provides adequate information about the structure of the heparinases as it relates to the functional role of the various regions of these enzymes as well as specific mutants thereof. These modified heparinases provide the needed "representative number" of species necessary to enable one of skill in the art to practice the invention.

It is respectfully requested that the rejection of claims 30, 33, 34, 46, 47 and 48 be withdrawn.

Rejection of claims 30, 31, 33, 46 and 47 Under 35 U.S.C. §102, (a)

Claims 30, 31, 33, 46, and 47 are rejected under 35 U.S.C. §102(a) as being anticipated by Shriver et al. (Journal of Biological Chemistry 273 (17): 10160-10167, April, 1998). The Applicants enclose herewith a Declaration under C.F.R. §1.131 to overcome this rejection.

Rejection of Claims 30, 33, 46 and 47 Under 35 U.S.C. §102, (e)

Claims 30, 33, 46, and 47 are rejected under 35 U.S.C. §102 as being anticipated by Su et al. (U.S. Patent No. 5,681,733). The Examiner maintains that these claims are anticipated by Su et al. as Su et al. discloses the isolation and sequence of the genes encoding heparinase II and III as well as the expression and substrate specificity in addition to k_{cat} for heparinase I, II and III. It is the Examiner's position that the claims are anticipated because the claimed enzymes differ only from the native enzymes described in Su et al. by functional attributes.

The claims are not anticipated by Su et al. because Su et al. does not describe each element of the claimed invention. The claims relate to methods for cleaving heparin or heparan sulfate like glycosaminoglycans using a class of modified enzymes. Su et al. does not disclose the production and/or use of modified heparinases having modified product profile or activity. The claims of the instant invention, however, provide modified heparinases that possess different properties than the native enzymes. The properties of interest as discussed in the specification include binding and catalytic function. Su et al., suggests that modifications to primary structure could be made but does not provide any guidance with respect to the preparation of such modifications. Su et al. does not make reference to modifying the heparinases to produce enzymes with altered function relative to the native enzymes and also does not disclose any information regarding the structure of the heparinases as it relates to enzymatic function. Since Su et al. does not describe the use of the claimed modified heparinases claims 30, 33, 46 and 47 are not anticipated by Su et al.

Rejection of Claims 34 and 48 Under 35 U.S.C. §103, (a)

Claims 34 and 48 are rejected under 35 U.S.C. §103(a) as being unpatentable over Su et al. as applied above to claims 30, 33, 46 and 47 and further in view of Langer et al. (U.S. Patent No. 4,373,023). It is the Examiner's position that one of ordinary skill in the art would have been motivated to use recombinantly produced heparinases such as those heparinases disclosed

in Su et al. with the method of Langer for cleaving heparin and removing heparin from blood by immobilized heparinase on solid supports. The Examiner maintains that there would have been motivation to use the heparinases isolated in Su et al. which can then be reproduced recombinantly. The Examiner further cites that it is well-known in the art of the advantages of recombinant production of proteins.

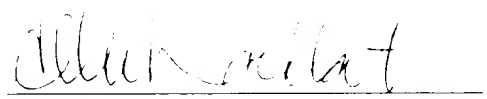
Even if one of skill in the art were to combine the teachings of Su et al. and Langer et al. as suggested, the combination would not result in the claimed invention. The claims require the production and use of modified heparinase II molecules having altered activities. As discussed above, the class of heparinases fully described by Su et al. is not the same class of enzymes claimed in the instant invention. Langer et al. does not describe the modified heparinases of the claimed invention. Neither reference provides any specific amino acid substitutions within recombinant heparinase II. Moreover, the references do not teach the methods and materials necessary to modify the sequences of the heparinases. Thus, even if the references were combined they would not have produced the claimed invention.

Summary

The Applicants believe that the above amendments and remarks are sufficient to place the claims in condition for allowance. If the Examiner disagrees, he is encouraged to contact the Applicants' representative to discuss any of the issues discussed above as well as any additional issues.

Respectfully submitted,

Sasisekharan et al.

By: 

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MARKED-UP CLAIMS

30. (Amended) A method of specifically cleaving a heparin-like glycosaminoglycan, comprising:

contacting a heparin-like glycosaminoglycan with the heparinase of any one of [claims 1, 8, or 22]

a substantially pure heparinase comprising a modified heparinase II having a modified product profile, wherein the modified product profile of the modified heparinase II is at least 10% different than a native product profile of a native heparinase II,

a substantially pure heparinase comprising a modified heparinase II that can cleave a glycosaminoglycan substrate having a modified heparinase II k_{cat} value, wherein the modified heparinase II k_{cat} value is at least 10% different than a native heparinase II k_{cat} value, and

a substantially pure heparinase comprising a modified heparinase I wherein the modified heparinase I has enzymatic activity that is not dependent on the presence of calcium.

33. (Amended) The method of claims 30, wherein the method is a method of removing [active] heparin from a heparin containing fluid.

46. (Amended) A method of specifically cleaving a heparan sulfate-like glycosaminoglycan, comprising:

contacting a heparan sulfate containing fluid with the heparinase of any one of [claims 1 or 8]

a substantially pure heparinase comprising a modified heparinase II having a modified product profile, wherein the modified product profile of the modified heparinase II is at least 10% different than a native product profile of a native heparinase II and

a substantially pure heparinase comprising a modified heparinase II that can cleave a glycosaminoglycan substrate having a modified heparinase II k_{cat} value, wherein the modified heparinase II k_{cat} value is at least 10% different than a native heparinase II k_{cat} value.

47. (Amended) The method of claim 46, wherein the method is a method of removing [active] heparan sulfate from a heparan sulfate containing fluid.